Molecular Pathogenesis of Malignant Mesothelioma
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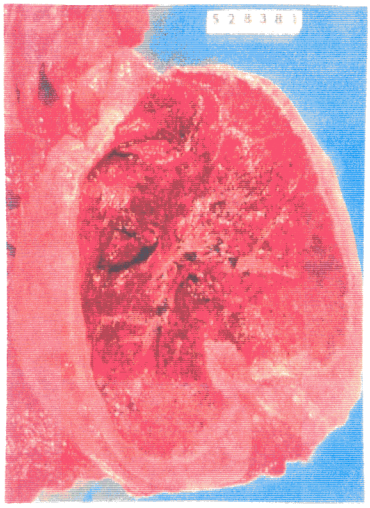
MALIGNANT MESOTHELIOMA



Jones, J. S. P. et al., Colour Atlas of Mesothelioma, MTP Press, 1985.

- rare: 2-20 cases / 10^6 / year
- more common with exposure to amphiboles
  chrysotile factories and mines may be contaminated with amphiboles
- latency of 15-60 years
- no association with cigarette smoking or asbestosis
- high incidence in shipbuilding and insulation industries
- difficult pathologic diagnosis
- poor response to therapy

OTHER CAUSES OF MESOTHELIOMA

Human:	irradiation
	chronic inflammation (tuberculosis)
	fibrous erionite (a hydrated aluminum silicate)
Animals:	iron chelates* (ferric saccharate)
	potassium bromate*
	fibrous erionite
	silicon carbide fibers
	refractory ceramic fibers
	E glass microfibers

*also cause kidney cancer

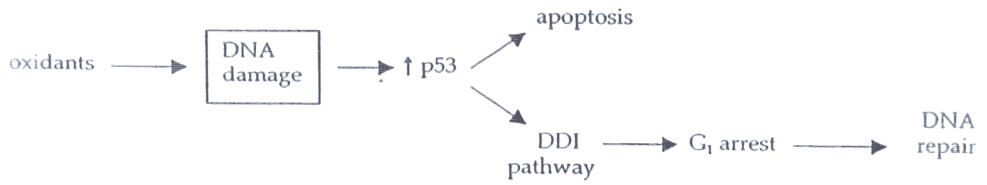
Mechanisms of Asbestos Carcinogenesis

Mechanism	Experimental End-Points	References
Genotoxic	Oxidized bases	Chao *et al.* (1966), Fung *et al.* (1997)
	DNA breaks	Okayasu *et al.* (1999)
	Aneuploidy	Reviewed in Jaurand (1996); Jensen *et al.* (1996)
	Mutations	Park & Aust (1998) Hei *et al.* (1995)
Non-genotoxic		
Mitogenic	Target cell proliferation	Bérubé *et al.* (1996); Goldberg *et al.* (1997); Mishra *et al.* (1997)
	Binding to or activation of surface receptors	Boylan *et al.* (1995); Pache *et al.* (1998)
	Growth factor expression	Liu *et al.* (1996); Brody *et al.* (1997); Kane *et al.* (1997)
	Intracellular signalling pathways	Zanella *et al.* (1996); Fung *et al.* (1997); Mossman *et al.* (1997)
Cytoxoxic	Apoptosis	Broaddus *et al.* (1996); Goldberg *et al.* (1997); Levresse *et al.* (1997)
	Necrosis	Reviewed in Kane (1996)

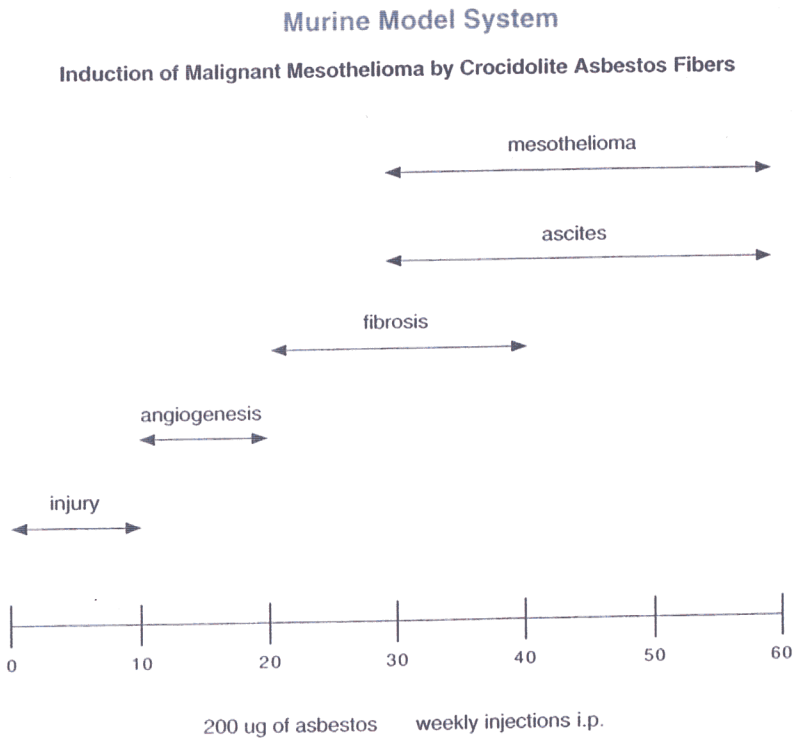
WORKING HYPOTHESIS

- A proposed mechanism for asbestos carcinogenicity is iron-catalyzed generation of free radicals that damage cellular DNA and induce oxidant stress.
- The p53 tumor suppressor gene product is important in mediating cell cycle arrest and DNA repair in response to DNA strand breaks
- A murine mesothelial cell line with a point mutation in p53 is defective in the G1 cell cycle checkpoint and shows increased sensitivity to asbestos genotoxicity (Cistulli et al., 1996).
- It is hypothesized that p53-deficient mice will show increased susceptibility to mesotheliomas induced by crocidolite asbestos fibers.

Cellular Responses to Oxidant-Induced DNA Damage



DDI – DNA damage inducible



A murine model system to study the acute and chronic effects of crocidolite asbestos fibers after direct intraperitoneal injection has been developed. The inflammatory and proliferative reactions to intraperitoneal injection of crocidolite asbestos fibers have been characterized in this model. Focal areas of mesothelial injury are repaired by proliferation of adjacent, uninjured cells after a single intraperitoneal injection of crocidolite asbestos fibers. These proliferating mesothelial cells are potential targets for genetic damage, induced directly by physical interference of fibers with the mitotic apparatus or indirectly by reactive oxygen and nitrogen metabolites released from inflammatory cells. The availability of iron at the surface of fibers is a critical parameter in catalyzing the generation of these highly reactive oxygen and nitrogen radicals. Ferric and ferrous cations are major components of amphibole asbestos fibers; iron may also be present as surface impurities on serpentine asbestos or some man-made fibers. Reactive oxygen and nitrogen metabolites can damage DNA through base mutations, DNA breaks, deletions, rearrangements, insertions, and altered patterns of methylation.

Increased sensitivity of p53-deficient mice to asbestos-induced genotoxicity

Figure 1

In-vivo Micronucleus Assay

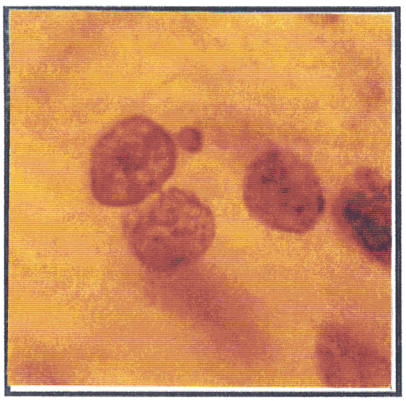


Table 1 Induction of Micronuclei in Proliferating Mesothelial Cells In Vivo

	% of Mesothelial Cells with Micronuclei	
Treatment	p53 +/+	p53 -/-
Saline	0.49 ± 0.49	0.28 ± 0.95
Asbestos	1.35 ± 0.05*	3.48 ± 0.52+
Wollastonite	0.76 ± 0.09	not tested

*p < 0.05 asbestos vs. saline-injected controls
+p < 0.05 p53 +/+ vs. p53 -/- mice

Malignant mesotheliomas induced by asbestos fibers in p53-deficient mice show decreased latency and increased invasion

Figure 2

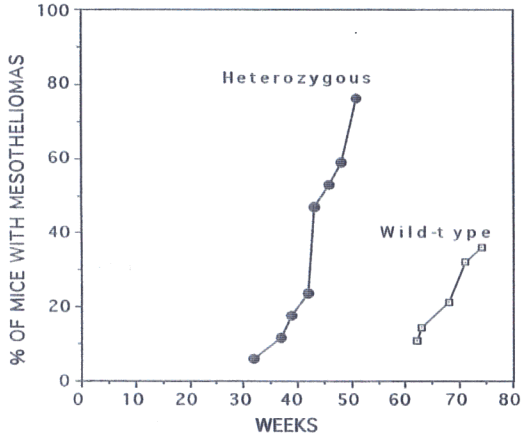


Figure 3 Papillary growth with spheroids

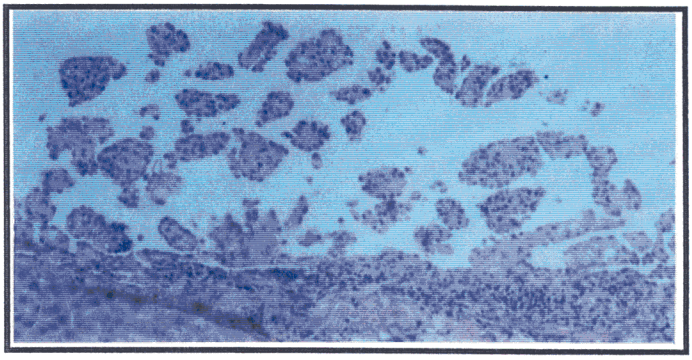


Figure 5 Lymphatic invasion

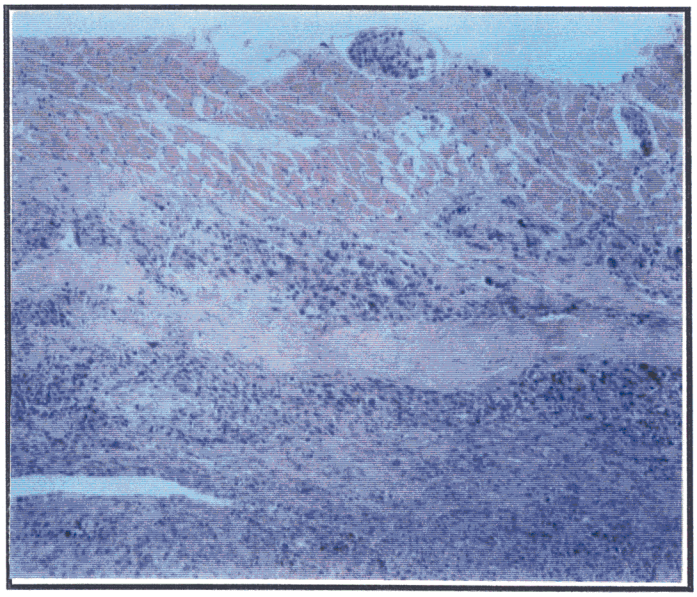
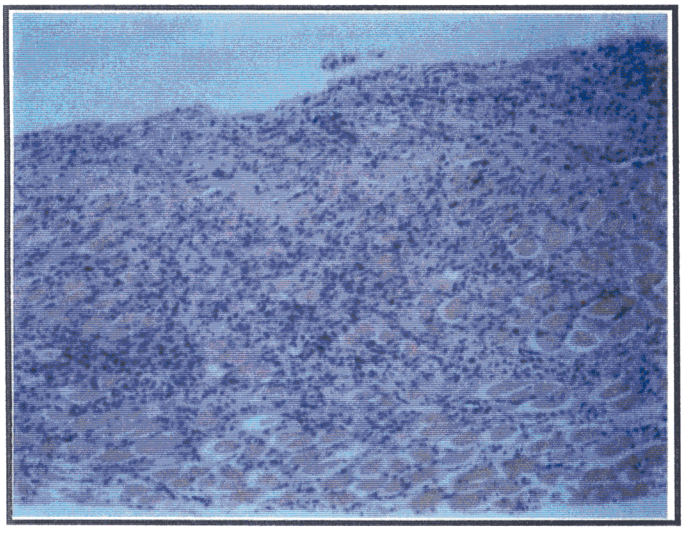


Figure 4 Local invasion into muscle



INACTIVATION OF p53 AND TUMOR PROGRESSION

- decreased DNA repair, genetic instability
- decreased apoptosis
- resistance to hypoxia
- increased angiogenesis

CLINICAL RELEVANCE

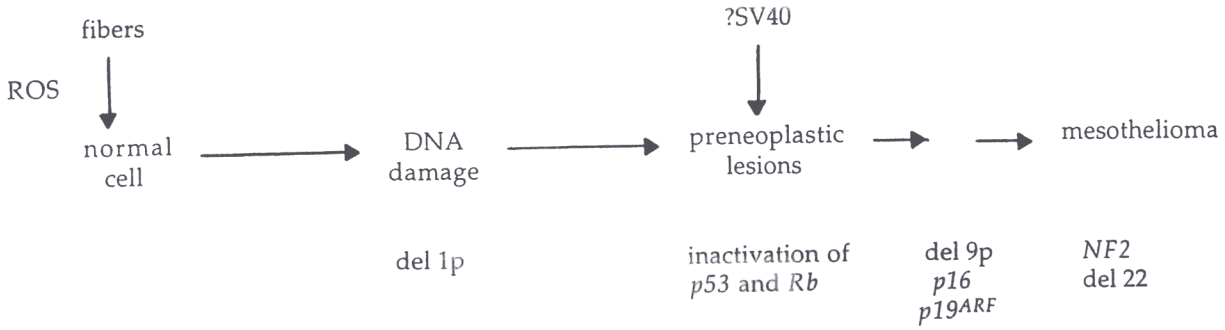
1. Patients with familial cancer syndromes (Wilms' tumor, Li-Fraumeni syndrome) develop mesothelioma after radiation therapy.
   Antman et al., Austin et al., 1986; Hisada et al., 1998

2. Slightly increased risk of mesothelioma in people exposed to asbestos who have first degree relatives with the Li-Fraumeni syndrome.
   Heineman et al., 1966

3. Point mutations and deletions in p53 are rare in human malignant mesotheliomas.
   Metcalf et al., 1992

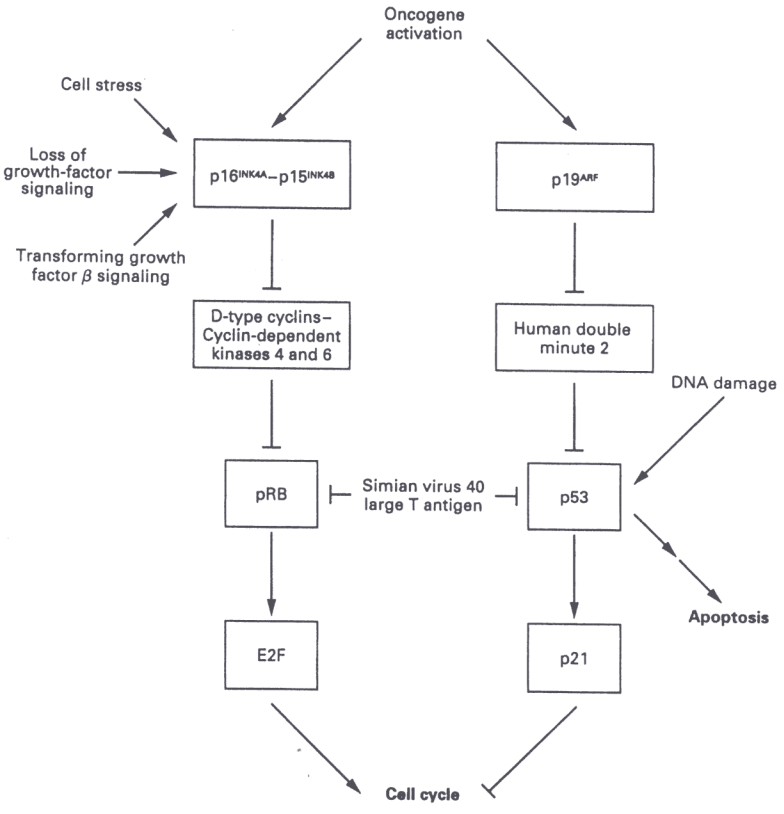
4. SV40 DNA sequences and T antigen have been identified in 60% of human malignant mesotheliomas.
   Carbone et al., 1999

MOLECULAR PATHOGENESIS OF HUMAN MALIGNANT MESOTHELIOMA



Murthy and Testa, J. Cell. Physiol. 180: 150-157, 1999

RETINOBLASTOMA PROTEIN AND p53 TUMOR SUPPRESSOR PATHWAYS



WC Hahn and RA Weinberg, New Engl J Med 347:1593, 2002

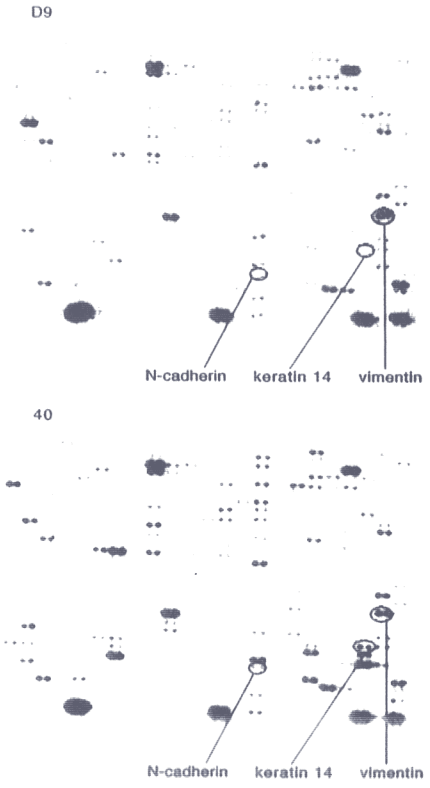
Asbestos Fibers and SV40 Virus as Co-Factors for Mesothelioma

(Summarized in Science 296: 1012, 2002; JNCI 94: 229, 2002)

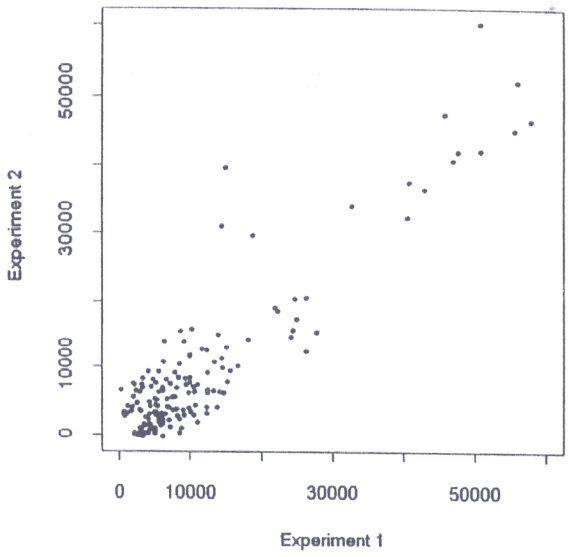
Evidence Against	Evidence For
Epidemiologic studies show no association between polio vaccine and increased risk of cancer - ? statistical power	SV40 virus causes mesothelioma in hamsters
	SV40 virus transforms human mesothelial cells in culture
What is the route of SV40 transmission in nonvaccinated people?	Multiple assays detect SV40 viral sequences in human mesotheliomas – PCR, immunohistochemistry, ISH
PCR assays are subject to cross-contamination	
Immunoassays cannot distinguish between SV40 virus and other human polyomaviruses – JC and BK	Antisense constructs against SV40 T antigen inhibit growth of human mesothelioma cells in culture
SV40 viral sequences are not found in all human mesotheliomas nor in all cells of the tumor	

GENE EXPRESSION PROFILE OF MURINE MESOTHELIOMA CELL LINES

cDNA Microarrays



Reproducibility of Gene Expression Profile



76% of genes are within a 2.5-fold range
Pearson correlation coefficient = 0.91

Summary

- cDNA microarrays confirm expression of mesothelial cell markers:

  cytokeratins, vimentin, CD44, N-cadherin

- Gene expression profile of murine malignant mesothelioma cell lines is consistent with molecular or immunohistochemical analysis of human mesotheliomas:

  N-ras, Bax, c-met, MCP-1, TGF-β, CSF-1, glutathione reductase, and glutathione-S-tranferases

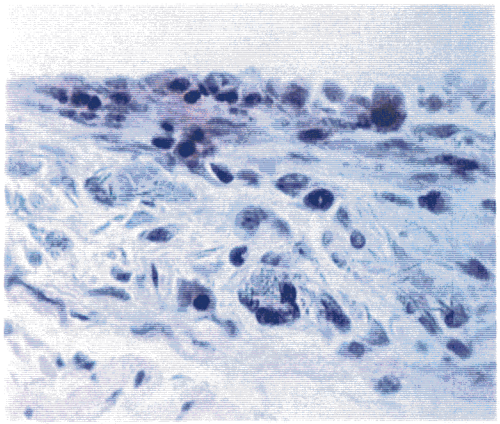
- Multiple functional pathways are altered in mesothelioma cell lines:

  activation of signaling pathways (PKC-δ, MAPK, p38)
  induction of early response genes (c-fos, c-jun, Egr-1)
  altered cell-cycle control
  resistance to apoptosis and expression of pro-survival genes increased motility

INFLAMMATION IN TUMOR PRORESSION

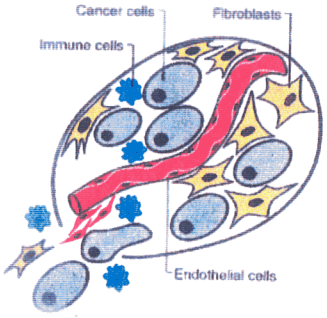
- Chronic inflammation predisposes to cancer-liver, stomach, urinary bladder, skin
- NSAIDs decrease risk of colon cancer-inhibition of COX-2 and prostaglandin synthesis
- Mice deficient in macrophages show decreased angiogenesis and slower tumor growth

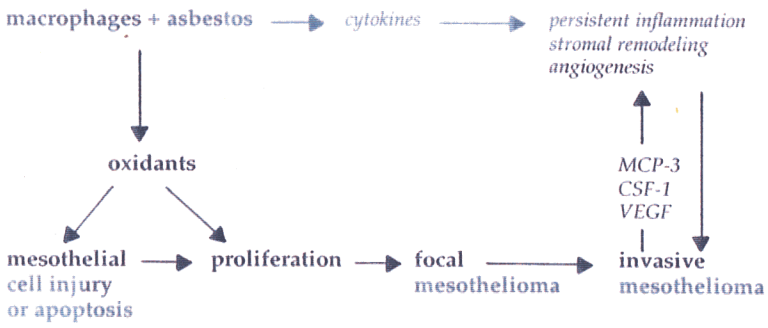
MACROPHAGES ARE THE INITIAL TARGET CELLS THAT INTERACT WITH FIBERS



The microenvironment of tumors consists of capillaries, fibroblasts, and inflammatory cells. In this murine model of mesothelioma produced by direct intraperitoneal injection of crocidolite asbestos fibers, long fibers are trapped at lymphatic openings on the inferior surface of the diaphragm and provoke focal accumulation of activated macrophages. In response to these biopersistent fibers, activated peritoneal macrophages express inflammatory cytokines (TNFα), cyclooxgenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and matrix metalloproteinases (MMP9, MMP12). Fiber clusters are surrounded by multinucleated giant cells and granulation tissue, similar to a healing wound. Diffuse malignant mesotheliomas frequently arise near these fiber clusters and infiltrate the underlying stroma. It is hypothesized that this chronic inflammatory environment facilitates growth and invasion of malignant mesotheliomas.

MACROPHAGES CONTRIBUTE TO PROGRESSION OF MALIGNANT MESOTHELIOMAS





D Hanahan and RA Weinberg,

The Hallmark of Cancer. Cell 100:57, 2000.